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**Recovery trends of phytoplankton and benthic macroinvertebrate communities  
in acidified and reference boreal lakes - a multihabitat assessment**

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Running head: Assessing recovery of boreal lakes

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## Summary

1. As a result of concerted international efforts to protect and restore natural resources threatened by acidification, rates of acid deposition have decreased markedly and widespread increases in surface water pH have been attributed to the improved air quality. Surface water chemistry exerts a major control on aquatic biodiversity, thus it is anticipated that improvement in surface water quality (e.g. raised pH) should result in biological recovery, albeit with inherent time lag responses, but records of biologic recovery are scarce so far.
2. Recovery can be defined as the movement towards a pre-acidified or reference state as stress decreases. Accordingly, finding no difference in water chemistry /or biology between previously acidified and reference lakes might be interpreted as a recovery from acidification.
3. This study intends to evaluate the effects of selection of organism group/trophic level and habitat on detecting recovery of lake ecosystems from acidification and to investigate the recovery rates of different indicators (water chemistry, biota), trophic levels (phytoplankton producers, macroinvertebrate consumers), and habitats (pelagic, benthic).
4. Several of the metrics analysed here showed significant, positive trends during the 16-year study period. Phytoplankton taxon richness and diversity recovered quickly and were strongly correlated to positive changes in pH in both reference and acidified lakes.
5. Littoral macroinvertebrate taxon richness and diversity also increased in reference and acidified lakes.

6. In contrast, sublittoral and profundal macroinvertebrate communities of both lake types showed clear negative trends, indicating that they may be more influenced by climate-related variables acting on habitat quality (e.g. ambient oxygen and temperature) than by changes in lake acidity. Moreover, this indicates that choice of not only organism but also habitat may be important in detecting biological recovery. Significant differences between acidified and reference lakes were obvious at the end of the study period, indicating that the acidified lakes have not fully recovered to the expected ecological targets.
7. *Synthesis and applications.* Our findings indicate that recovery is a complex process influenced by multiple spatial and temporal factors and suggest the use of multiple organism groups and trophic levels to better detect and understand the processes and changes within the lake ecosystem.

## Introduction

International agreements and actions to protect and restore natural resources threatened by acidification have resulted in reductions in both the emission and deposition of acidifying compounds across western Europe and eastern North America (e.g. Stoddard *et al.* 1999). As a result of these concerted international efforts, rates of acid deposition have decreased markedly and widespread increases in surface water pH have been attributed to the improved air quality (e.g. Stoddard *et al.* 1999; Skjelkvåle *et al.* 2000; Lynch *et al.* 2000; Skjelkvåle *et al.* 2003). Because surface water chemistry exerts a major control on aquatic biodiversity (e.g. Resh & Rosenberg 1993), it is anticipated that improvement in surface water quality (e.g. raised pH) should result in biological recovery, albeit with inherent time lag responses (Evans *et al.* 2001). However, in contrast to the growing literature documenting improvement of surface water pH, records of biological recovery are scarce and results are equivocal (Skjelkvåle *et al.* 2000; Alewell *et al.* 2001) and acidification is still considered one of the foremost problems affecting the biodiversity of inland surface waters in northern Europe (e.g. Brodin 1995; Johnson *et al.* 2003). Biological recovery has been related to chemical recovery in some lakes and streams, but improved biota has not been observed with better water quality in others. In the UK and Norway, changes in invertebrate communities from acidophilic to acid-sensitive taxa and increases in trout density have been observed (Raddum *et al.* 2001; Tipping *et al.* 2002) and acknowledged as a response to increased pH. In contrast, although invertebrate recovery has been recognized in two Swedish lakes (Halvorsen *et al.* 2002), evidence of broad-scale, biological recovery of acid-stressed lakes in Sweden is still lacking.

A number of factors may confound our ability to detect ecological recovery. Yan *et al.* (2003) recognized, for example, three bottlenecks that may delay biological recovery: inadequate water quality, inadequate supply of colonists to permit establishment and community-level impediments to recovery dynamics. Similarly, Wright (2002) hypothesized that the choice of indicator organism/trophic level as well as habitat may confound assessments of chemical and biological recovery. Site-specific factors such as latitude, altitude, catchment characteristics (e.g. soil/bedrock type, land use/cover), ecosystem size, and nutrient status may result in different lag responses. For example, Wright (2002) postulated that lag responses may increase with trophic level (e.g. changes or instability at a higher trophic level may affect lower trophic levels), and are generally longer and more variable for systems with long water retention times (e.g. lakes) compared to systems with relatively short retention times (e.g. streams).

Acidification is often characterized by marked decreases in pH, alkalinity/acidity and acid-sensitive species and increases in SO<sub>4</sub> concentration and acid-tolerant species. Acidification often results in relatively predictable changes in species composition, with the loss of acid sensitive species and increases in the abundance of acid tolerant species. For example, the number and diversity of pelagic and benthic species have been shown to be strongly related to pH, but also habitat diversity and nutrient level (e.g. Økland & Økland 1986; Appelberg & Degerman 1991). At pH < 5.0, phytoplankton communities are often predominated by chlorophyceans, chrysophceans (e.g. *Dinobryon*) and pyrrophytes (e.g. *Peridinium* and *Gymnodinium*) (e.g. Almer *et al.* 1974; Degerman *et al.* 1995). Similarly, marked changes have also been observed for

benthic communities, with snails (Gastropoda), mussels (Bivalvia), amphipods and mayflies (Ephemeroptera) often showing decreases in taxon richness and diversity, whilst other groups (e.g. Corixidae and *Notonecta*) often increase in abundance. Although acidification has been shown to affect lake communities in a predictable way (through both abiotic and biotic pathways), the underlying processes and mechanisms are not always clearly understood (e.g. Hildrew & Ormerod 1995). Moreover, a number of factors like connectivity to source populations and dispersal ability, food availability and species interactions (presence of predators and competitors) may individually or in concert affect rates and trajectories of biological recovery resulting in hysteresis.

With the declines in sulphur deposition, inland waters are now globally recovering from acidification (Evans et al. 2001) and more focus is now being placed on understanding the factors associated with biological recovery. Recovery can be defined as the movement towards a pre-acidified or reference state as stress decreases (Wright 2002). For ecological targets, a number of previous studies on boreal Swedish lakes have assumed minimally impacted reference lakes with a  $\text{pH} > 6$  to be representative of the expected, “natural” state (Johnson 2005; Naturvårdsverket 2000). Accordingly, finding no difference in water chemistry and/or biology (e.g. species composition, diversity and richness) between acidified and reference lakes has been interpreted as evidence that lakes have recovered from acidification. This conjecture recognizes that natural communities are not static but exhibit among-year variability. Use of a space-for-time, reference condition approach often assumes that short-term variability should fluctuate around a long-term mean, but that in the long-term no trends should be evident. Unfortunately, to our knowledge few

studies have compared and contrasted the rates and trajectories of recovery with those of natural temporal variability of reference sites. Another factor that needs considering when working with long-term trends is the effect that changes in global climate will have on both reference and restored sites, such as higher variability in the short term and possibly altered steady states in the long term.

In this study, we were interested in testing the effects that selection of organism group/trophic level and habitat might have on detecting recovery of lake ecosystems from acidification. More specifically, we were interested in studying the recovery rates of different indicators (water chemistry, biota), different trophic levels (phytoplankton producers, macroinvertebrate consumers), and habitats (pelagic, benthic). (1) Firstly, we expected that chemical recovery would be detected, albeit with acidic episodes, more quickly than biological recovery. This assumption is based on the increasing number of studies showing chemical recovery following reductions in acid deposition (e.g. Stoddard *et al.* 1999; Skjelkvåle *et al.* 2003). (2) Cognizant that chemical recovery is often associated with periods of high acidity (e.g. episodic low pH events during spring runoff) we expected that biological response would vary with organism group and trophic level. We selected phytoplankton (a primary producer) and littoral benthic macroinvertebrates (a consumer) to test for early signs of recovery. Although both phytoplankton and littoral macroinvertebrates are expected to respond quickly to reductions in acid stress, we anticipated that the response time and effects of episodic low pH events would be more deleterious for littoral macroinvertebrates than for phytoplankton due to a longer generation times for invertebrates. Accordingly, we hypothesized that recovery rates would be shorter for

phytoplankton than for littoral macroinvertebrates. This assumption is based on the shorter generation times and higher re-colonization and dispersal rates (seed banks and more easily dispersed by wind and waterfowl) of phytoplankton. (3) Thirdly, we tested the hypothesis that habitat may confound interpretation of recovery effects by analysing organism response in three benthic habitats. Here, we postulate that littoral communities should respond more quickly to changes in surface water chemistry (changes in acidity and associated co-variables) than either sublittoral or profundal communities. Sublittoral and profundal communities, although not directly affected by surface water chemistry, may respond to changes in food (bottom up) or predation (top down), both of which may result in longer lag phases. (4) Finally, since ecosystems are not static but are expected to change over time, we were also interested in determining the importance of changes in large-scale climatic factors for the rates and trajectories of change in acidified as well as spatial reference sites.

## **Methods**

### *Study sites and sampling*

In the late 1980s, Sweden initiated a long-term monitoring program of multiple habitats and trophic levels to follow the effects of acidification and recovery of regionally representative lake ecosystems (Johnson 1999; Wiederholm & Johnson 1997). Two lake categories were selected: (i) non-acidified 'reference' lakes to monitor natural, among-year changes and (ii) acidified lakes (> 0 exceedence of critical load of S) to monitor natural recovery from acid stress following implementation of international agreements to reduce acidic emissions.

Water chemistry, phytoplankton and macroinvertebrate communities were sampled from ten lakes distributed over Sweden (Fig.1). The dataset analysed here consists of 16 consecutive years from 1988 until 2003. Six lakes with a mean pH > 6 are used here as minimally disturbed reference lakes and four lakes with a mean pH < 6 are considered as anthropogenically acidified (estimation of exchange of preindustrial alkalinity versus anthropogenic sulfate ions calculated by Wilander (1994) (Persson 1996). All ten lakes are relatively small (mean lake area 0.62 km<sup>2</sup> for reference and 0.56 km<sup>2</sup> for acidified), shallow (mean depth 5.7 m for reference and 5.02 m for acidified), nutrient poor and clear-water lakes (see Table 1 & 2). Lake catchments were also similar between reference and acidified lakes; percent forest averaged 79.9 and ranged from 40.2 to 88.6 for reference lakes and from 79.8 to 96.8 for acidified lakes. Of the water chemistry shown in Table 1, pH, alkalinity/acidity, total nitrogen as well as water color differed between reference and acidified lakes (Table 2). More information on these lakes is available on <http://www.ma.slu.se>.

Surface water samples (0-2 m) were collected six to eight times a year by taking a mid lake water sample using a Plexiglas sampler and were kept cool during transport to the laboratory. Water samples were analysed for variables indicative of acidity (e.g. pH and alkalinity/acidity), nutrients (e.g. total phosphorus and nitrogen), conductivity and colour (absorbance of filtered water). All physico-chemical analyses were done at the Department of Environmental Assessment following international (ISO) or European (EN) standards when available (Wilander *et al.* 2003). For statistical analyses, annual mean values were taken to down-weight seasonal differences.

Phytoplankton was sampled in August of each year by taking a water sample from the epilimnion (0-4 m) using a Plexiglas tube sampler (diameter = 3cm). In lakes with a surface area  $> 1 \text{ km}^2$  a single mid-lake site was used for sampling. In lakes with a surface area  $< 1 \text{ km}^2$ , five random epilimnion water samples were taken and mixed to a composite sample from which a subsample was taken and preserved with JJK-solution supplemented with acetic acid. Phytoplankton counts were made using an inverted light microscope and the Utermöhl technique modified and commonly used in the Nordic countries (Olrik *et al.* 1989). Taxonomic resolution was done to the lowest taxonomic unit possible (usually species). However, here the total biovolume and biovolume of seven groups (Cyanophyceae, Cryptophyceae, Dinophyceae, Chrysophyceae, Bacillariophyceae, Chlorophyceae, miscellaneous species (i.e. low abundant species with low biovolume not belonging to these groups) were used to calculate taxon richness and Simpson diversity. Taxon richness refers to the number of groups present in one sample/year; Simpson diversity (D) (Simpson 1949) was calculated in CANOCO for Windows Version 4.5 (ter Braak & Smilauer 1997-2002) using the Hill's scaling function on inter-sample distances.

Benthic macroinvertebrates of three habitats were collected in late autumn (October – November) each year. Littoral samples were collected using standardized kick-sampling (European Committee for Standardization 1994) with a handnet (0.5 mm mesh size). A composite sample consisting of five kick-samples (20 sec x 1 m ca 0.5 m depth) was taken from hard bottom, vegetation-free sites of each lake. Sublittoral samples consisted of five replicate Ekman samples (ca  $247 \text{ cm}^2$ ) taken within a 50 x 100 m rectangular in 4-6 m depth. Sublittoral regions were

defined as areas lying under the late-summer thermocline in stratified lakes. Profundal samples consisted of five replicate Ekman samples (ca 247 cm<sup>2</sup>) taken within a 150 x 150 m quadrat within the deepest area of the lake. Macroinvertebrate samples were preserved in 70% ethanol in the field and in the laboratory the samples were processed by sorting under 10x magnification, identified and counted using dissecting and light microscopy. Organisms were identified to the lowest taxonomic unit possible, generally to species level.

Three metrics were used to analyse for changes in phytoplankton and benthic macroinvertebrate communities. Taxon richness was selected as a qualitative measure of changes in community composition. Community diversity was calculated as Simpson's diversity (Simpson 1949) in CANOCO for Windows Version 4.5 (ter Braak & Smilauer 1997-2002) using the Hill's scaling function on inter-sample distances. The first axis scores of correspondence analysis (CA axis 1 scores) were used to represent community composition (i.e. the relative abundance of taxonomic composition).

### *Statistical analyses*

#### *Comparison of reference and acidified lakes – space for time*

Analyses of variance (one-way ANOVA) and two-sided t-tests were conducted to obtain differences between the reference and acidified lakes as well as within reference and acidified lakes between years. Separate analyses were run for water chemistry, phytoplankton and benthic macroinvertebrates (each of the three habitats) to test i) differences between reference and acidified lakes over all years (grand mean of the chemical and biological variables), ii) to test for

differences in chemical and biological variables between reference and acidified lakes for the first (1988) and the last year (2003) of the study, and iii) to test for differences in chemical and biological variables between the first (1988) and the last year (2003) of the study within the reference and within the acidified lakes.

*Comparison of reference and acidified lakes – regression*

Linear regression of annual mean water chemistry and biological metrics (CA axis 1 scores, taxon richness and Simpson diversity (Simpson 1949) against time was used to determine if communities are showing signs of recovery. Separate analyses were run for water chemistry, phytoplankton and benthic macroinvertebrates (each of the three habitats) for each lake.

Analysis of variance (ANOVA) of metric regression slopes and coefficients of determination ( $R^2$ ) was used to test for differences in trends between lake type (reference and acidified), organism group (phytoplankton and benthic macroinvertebrates) and habitats (pelagic, littoral, sublittoral and profundal) with the Tukey-Kramer HSD test (Kramer 1956; Tukey 1949).

*Comparison of reference and acidified lakes – correlative analysis of changes*

Stepwise multiple regressions (with forward selection) with pH as the dependent variable and water chemistry (i.e. Ca, Mg, alkalinity/acidity,  $\text{SO}_4$  deposition,  $\text{SO}_4$  (water), Cl and water colour) as the independent variables was conducted to determine the best predictor of changes in pH in both reference and acidified lakes. Similarly, stepwise multiple regressions, with forward selection, of phytoplankton and macroinvertebrate CA axis 1 scores, taxon richness and Simpson

diversity as dependent and surface water chemistry (pH, alkalinity/acidity, conductivity, TP, TN, water colour, water temperature) and the North Atlantic Oscillation winter index (NAO winter index from December through March, taken from the homepage of the National Center for Atmospheric Research, U.S.; <http://www.cgd.ucar.edu/cas/jhurrell/nao.stat.winter.html>) were conducted to determine the best predictors of changes in community composition. The number of significant relations within each lake group and habitat were combined resulting in a total number of times the specific variable was selected.

Correspondence analyses were made with CANOCO for Windows Version 4.5 (ter Braak & Smilauer 1997-2002). Linear regressions, ANOVAs and multiple regressions were performed using the statistical program JMP (SAS 1994). Prior to analyses, phytoplankton biovolume and chemistry variables were  $\log_{10}$ - and benthic fauna abundance data were square-root-transformed to approximate normal distributions.

## **Results**

### *Comparison of reference and acidified lakes – space for time*

A comparison of selected water chemistry variables between reference and acidified lakes revealed a number of differences. For example, the acidity metrics pH and alkalinity/acidity as well as TN concentration and water color differed between the reference and acidified lakes (Table 2). Both acidity metrics also differed between the first (1988) and last (2003) years of the 16-year study. However, only pH differed significantly between the first and last year for

reference lakes. Similarly, clear differences were noted for lake biology. With the exception of lake profundal macroinvertebrate taxon richness and diversity, all other biological metrics differed between reference and acidified lakes. However, only phytoplankton differed between the start and end of the study period, whereas littoral macroinvertebrate diversity differed only in 1988. In contrast to lake water chemistry, all biological metrics, with the exception of sublittoral macroinvertebrate diversity, showed significant differences in reference lakes between 1988 and 2003. For acidified lakes, only littoral macroinvertebrate diversity differed between the two years.

#### *Comparison of reference and acidified lakes – regression*

##### *Water chemistry*

Water column pH increased in both reference and acidified lakes during the 16-year sampling period. Moreover, stepwise multiple regressions of pH against other chemical variables showed that SO<sub>4</sub> deposition was often (in 50% of all lakes) selected as the best predictor of changes in pH. However, although pH increased significantly in several of the study lakes, several of the acidified lakes had pH values below biological thresholds (grand mean =  $5.22 \pm 0.42$  for acidified;  $6.49 \pm 0.19$  for reference lakes) (Table 1 & 2). Among-year variability (median R<sup>2</sup>) of pH, conductivity and water color was similar between reference and acidified lakes (Table 2) (Fig. 2b, d, e). Linear regression revealed significant positive trends of pH in four of the six reference and in three of the four acidified lakes; median pH increased by 0.008 units year<sup>-1</sup> in the reference and by 0.024 units year<sup>-1</sup> in the acidified lakes (Fig. 2a). Many of the selected chemistry variables either decreased or increased during the study period, in both reference as well as

acidified lakes. Conductivity and total phosphorus (except for L. Fiolen) decreased markedly in both reference and acidified lakes. Decreases in conductivity were significantly more pronounced in the acidified than in the reference lakes (Table 2) (Fig. 2c). Total nitrogen and water color were generally inversely related to changes in conductivity. Total nitrogen increased in three reference lakes, but showed negative, although non-significant, trends in three of the four acidified lakes (except for L. Brunnsjön). Water color increased in both reference ( $n = 5$ ) and acidified ( $n = 3$ ) lakes ( $p < 0.05$ ), but changes were more pronounced in acidified lakes (Fig. 2e). Except for conductivity slopes, no differences were noted between the slopes or among-year variability of pH, conductivity or water color in reference and acidified lakes (Tukey-Kramer HSD,  $p < 0.05$ )

### *Phytoplankton*

The number of phytoplankton taxa increased in three reference and all four acidified lakes during the 16-year study period (linear regression,  $p < 0.05$ ) (Table 3). Similarly, diversity increased in five reference and three acidified lakes (linear regression,  $p < 0.05$ ). Regression slopes of taxon richness against time were significantly different between reference and acidified lakes (Table 3) (Fig. 3a). Conversely, among-year variability in taxon richness was on average lower for reference than for acidified, but reference lakes showed a greater range in variability (Table 3) (Fig. 3b). In contrast to differences between slopes of taxon richness in reference and acidified lakes, no differences were noted regarding diversity (Fig. 3c) or among-year variability of diversity between reference and acidified lakes (Fig. 3d).

Several phytoplankton families contributed to the increase in taxon richness and diversity of reference lakes. Crysophyceae and Chlorophyceae increased in biovolume in five of the six reference lakes, while Cyanophyceae and Cryptophyceae increased in biovolume in four (Table 4). By contrast, Chlorophyceae increased in biovolume in only one of the four acidified lakes, while Cryptophyceae increased in biovolume in three lakes.

### *Macroinvertebrates*

Both littoral taxon richness and diversity of reference lakes showed positive trends during the study period (Fig. 4). Taxon richness of littoral habitats increased in three of the six reference lakes; in two lakes (L. Fiolen and L. Fräcksjön) richness increased by nearly one taxon year<sup>-1</sup> (median slope for all six reference lakes = 0.97 taxon year<sup>-1</sup>) (Table 5) (Fig. 4a). Likewise, diversity of littoral habitats showed positive trends in four of the six reference (median slope for all six reference lakes = 0.52 diversity units year<sup>-1</sup>) (Fig. 4c). Among-year variability in taxon richness was markedly lower than that for diversity (median  $R^2 = 0.15$  and 0.24, respectively) (Fig. 4b & d).

By contrast, taxon richness and diversity of both sublittoral and profundal habitats showed strong, negative trends (Table 5) (Fig. 4a & c). Sublittoral macroinvertebrate richness decreased markedly in four of the six reference lakes (mean slope = -0.655 taxon year<sup>-1</sup>); two lakes had regression slopes indicating > one taxon year<sup>-1</sup> (L. Fiolen and L. Stora Skärsjön) (Table 5). Taxon loss also resulted in pronounced decreases of diversity in three reference lakes. Similar to the trends noted for sublittoral communities, taxon richness of profundal habitats decreased

significantly in five reference lakes. However, trends in diversity were not as marked; only two of the six reference lakes showed significant decreases in diversity. Only one lake (L. Fiolen) showed positive trends in taxon richness (slope =  $0.44 \text{ taxon year}^{-1}$ ,  $p < 0.01$ ). However, the loss of taxon richness was not reflected in changes in diversity ( $p > 0.05$ ). In contrast to littoral habitats, among-year variability (median  $R^2$ ) in taxon richness was higher than that for diversity (= 0.34 and 0.28, respectively, for sublittoral communities), but no markedly differences were noted between sublittoral and profundal habitats (Fig. 4b & d).

Temporal trends of taxon richness and diversity in the four acidified lakes were similar to those observed for reference lakes. Significant increases of both littoral taxon richness and diversity, indicating biological recovery, were noted in all four of the acidified lakes (Table 5). On average, littoral communities gained nearly one taxon  $\text{year}^{-1}$  during the 16-year study period (median slope =  $0.88 \text{ taxon year}^{-1}$ ). Two lakes, in particular, L. Brunnsjön and L. Rotehogstjärnen had slopes  $> 1 \text{ taxon year}^{-1}$ . However, among-year variability in taxon richness and diversity were not significantly different between acidified and reference lakes (Fig. 4b & d). In contrast to reference lakes, negative trends in taxon richness and diversity for sublittoral or profundal communities were not evident (median slopes, Table 5) (Fig. 4a & c). Only one lake (L. Härsvatten) showed a significant increase in sublittoral taxon richness (slope =  $0.4 \text{ taxon year}^{-1}$ ,  $p < 0.05$ ) and one lake (L. Rotehogstjärnen) showed a significant decrease of profundal diversity (slope =  $-0.14 \text{ diversity units year}^{-1}$ ) (Table 5).

In contrast to increases in phytoplankton taxon richness, few macroinvertebrate taxa showed significant population increases in more than a few lakes. Thirteen taxa were found to increase significantly in abundance (calculated from the top ten from each lake) in littoral habitats of reference lakes (Table 6). However, only three taxa were found to increase in more than one lake (i.e. the mollusc *Pisidium* showed significant population increases in three lakes, the mayfly *Caenis horaria* and the chironomid midge increased in two). By contrast, all but one taxon (*Sialis lutaria*) decreased in sublittoral habitats; five taxa decreased in more than one lake. Populations of the isopod *Asellus aquaticus* decreased in four of the six reference lakes, followed by the chironomid midge *Pagastiella orophila* and *Pisidium*, which decreased in three lakes, and the chironomid midges *Procladius* and *Zalutschia zalutschicola*, which decreased in two lakes. Only seven taxa were found to decrease or increase in profundal habitats. One taxon increased (the phantom midge *Chaoborus flavicans*), while the other five showed population decreases (e.g. *Pisidium* decreased in two reference lakes). Similar to the trends noted for reference lakes, all but one taxon (*Sialis sp.*) increased in littoral habitats, but only one taxon (*Asellus aquaticus*) increased in more than one acidified lake. In contrast to the negative trends noted for reference lakes, all but one taxon (*Sialis sp.*) showed increased population densities; however, no taxon occurred in more than one lake. Similar to profundal habitats of reference lakes, all but one taxon (*Chaoborus flavicans*) showed population decreases in acidified lakes.

#### *Comparison of metric/habitat response*

Regression slopes of the selected acidity (pH and alkalinity/acidity) and biological metrics (taxon richness and diversity) differed markedly, although with the exception of sublittoral and

profundal macroinvertebrate communities, similar patterns were noted between acidified and reference lakes (Table 7). Our prediction that water chemistry would show earlier signs of recovery than pelagic phytoplankton was not supported here. No differences were found between the slopes of pH (mean slope = 0.218 units year<sup>-1</sup>) or alkalinity/acidity (-0.0014 meqL<sup>-1</sup> year<sup>-1</sup>) and phytoplankton taxon richness (0.138 taxa year<sup>-1</sup>) or diversity (0.043 diversity units year<sup>-1</sup>). Indeed, contrary to our prediction, littoral macroinvertebrate communities showed the strongest response to improved water quality. Slopes of taxon richness and diversity averaged 0.845 taxa year<sup>-1</sup> and 0.488 diversity units year<sup>-1</sup>. Hence, our third prediction that littoral macroinvertebrate community response would be > sublittoral > profundal was only partly supported here; littoral slopes of taxon richness and diversity were > sublittoral, but no difference was noted between sublittoral and profundal communities.

*Comparison of reference and acidified lakes – correlative analysis of changes*

Stepwise regression of CA axis 1 scores, diversity and taxon richness as dependent variables and eight physico-chemical explanatory variables revealed a number of variables that were correlated with the temporal changes of the pelagic and benthic communities (Table 8). Both similarities and differences were noted regarding the “best” predictors between reference and acidified lakes and between habitats studied. For example, pH was single best predictor of the temporal changes in phytoplankton assemblages (n = 12), followed by conductivity (n = 11) and temperature (n = 10). Similarly, pH was single best predictor of the temporal changes in the littoral macroinvertebrate communities (n = 16), followed by temperature (n = 11). Regarding pH, no differences were noted between reference and acidified lakes. For sublittoral and profundal

communities, pH was seemingly less important ( $n = 9$  and  $10$ , respectively). Instead, water color (sublittoral) and TN (profundal) were shown to be best correlated with the among-year changes (water colour,  $n = 15$  for sublittoral and TN,  $n = 15$  for profundal).

## **Discussion**

Comparison of selected water chemistry and biological variables in boreal lake ecosystems indicated that biological recovery from acidification is a complex and challenging process, often requiring holistic and multiple approaches. Several of the metrics analysed here showed significant positive trends during the 16-year study. However, significant differences between the four acidified and six reference lakes were still noted at the end of the study period, indicating that the acidified lakes have not fully recovered to the expected ecological targets. For example, diversity of littoral macroinvertebrate communities has increased significantly but values are still below those expected from the reference lakes. These findings support the contention that recovery may vary depending on the indicator/habitat selected.

We found that phytoplankton communities recovered quickly in response to raised pH, and that the temporal trends in phytoplankton taxon richness and diversity were strongly correlated to changes in pH in both reference and acidified lakes. These findings were not unexpected, and support earlier work that has shown recovery of phytoplankton communities within 1 – 4 years after chemical recovery (e.g. Wright 2002). However, although phytoplankton is expected to recover quickly following improvements in water quality due to their rapid turnover times,

dispersion to new habitats and large seed banks, biological recovery may lag behind chemical recovery (e.g. Findlay 2003). Several factors such as regional-level impoverished diversity, inputs of toxic metals and inter-specific interactions may confound the establishment of circumneutral communities. Findlay (2003) showed, for instance, that natural chrysophyte species became dominant only after a decline of acid tolerant species.

A number of factors confound our understanding of biological recovery (Yan *et al.* 2003). In streams, the effect of continued episodic acidification events during a transition phase has been shown to affect biological recovery (e.g. Lepori *et al.* 2003b; Hirst *et al.* 2004). Likewise, acidic events during snowmelt or rain periods may continue to affect lake biotic seemingly decoupled from declining trends in acid deposition. For example, aquatic ecosystems situated in glaciated and mountainous areas are generally poorly buffered against acid deposition and more prone to rapid acidification due to thin soils, low cation exchange capacity and low retention of deposited sulphur. Conversely, in areas with older, deeper soils, lakes and streams generally show high buffering capacity and are acidified more slowly (Skjelkvåle *et al.* 2003). In the former, thin-soil example recovery is expected to occur rapidly (i.e. the inverse of acidification), whereas in the deep-soil example, recovery is expected to occur less quickly due to continued release of stored sulphur, which may even continue to acidify despite reduced acid deposition. For instance, in Germany, decreased sulphur deposition over the last decade has not resulted in decreased sulfate in runoff, due to sulphur leakage from the soil (Prechtel *et al.* 2001). Not only soil thickness, but also climate-related factors such as the frequency and severity of sea-salt episodes and droughts or an increase of organic carbon and nitrogen mineralization may obscure or confound trends in

recovery (Skjelkvåle *et al.* 2003). For example, high N deposition has recently been postulated to affect chemical recovery in streams (Evans & Jenkins 2000), resulting in episodic acidification (e.g. Stoddard 1991; Lepori *et al.* 2003a) and subsequently affecting biological recovery (Lepori *et al.* 2003b).

However, an increase in pH alone does not necessarily imply adequate water quality, as many other factors either alone or combined may restrict biological recovery. For example, although pH increased in the acidified lakes during the 16-year study period, it was still below the biological threshold of many organisms (mean pH was  $< 6$  in all four acidified lakes studied here). Nutrient levels also changed over the study period. Concentrations of total phosphorous, though relatively low in both reference and acidified lakes (generally  $< 10 \mu\text{g/L}$ ) at the beginning of the study (1988), decreased significantly in three reference and two acidified lakes. In contrast, nitrogen levels increased, which may be related to changes in water color (positive correlation between these two variables,  $p < 0.0001$ ). A recent study by Weyhenmeyer *et al.* (2005) has suggested that phytoplankton of boreal lakes might be N limited. Hence, increased inputs of N might have resulted in increased pelagic primary productivity and shifts in community composition over the study period. Increases in either pelagic (phytoplankton) or benthic (periphyton) production might explain the negative trends noted in sublittoral taxon richness of reference lakes.

Similar to pelagic phytoplankton communities, a number of internal and external processes may affect the establishment of benthic species and subsequently the shift from acid-tolerant to pre-disturbance acid-sensitive communities. Our finding that littoral macroinvertebrate taxon richness

and diversity increased in reference and acidified lakes during the 16-year study period indicates biological recovery, thus supporting the trends noted for phytoplankton assemblages. Recent studies of recovery of lake-littoral and stream-riffle macroinvertebrate communities in acidified surface waters in Scandinavia have shown similar trends (e. g. Raddum *et al.* 2001; Halvorsen *et al.* 2002). For instance, Raddum *et al.* (2001) noted an increased distribution of acid-sensitive invertebrates across some Norwegian rivers. In contrast, the finding that sublittoral and profundal macroinvertebrate communities of both lake types showed clear negative trends, was somewhat disconcerting but supports recent work on trends in boreal lakes (e.g. Johnson 2005). Our finding that reference communities are not fluctuating around a long-term mean as anticipated, but showing signs of degradation that is not coupled to site-specific stressors, implies the influence of large-scale, regional effects such as climate.

Several factors may explain the changes noted in sublittoral and profundal richness and community composition. As mentioned above, one plausible scenario explaining the negative trends in sublittoral and profundal taxon richness of reference lakes is the importance of bottom up processes. Pelagic phytoplankton or periphyton might be P-limited due to low phosphorous levels in these lakes and thus (further) decreases in P may affect primary produces and ultimately collector-grazer populations. Our finding that negative trends in sublittoral and profundal communities were correlated with increasing water colour, TN concentration and decreasing conductivity might be related to changes in climate such as the unusual warmer and wetter winters during the early 1990s as indicated by high  $NAO_{winter}$  indices. During wet periods, increased surface runoff and higher inputs of dissolved organic matter (water colour), might

negatively affect primary productivity and food availability due to shading. As mentioned above, high N input might also result in increased primary productivity, which together with high water colour might negatively affect sublittoral and profundal communities by decreases in oxygen concentration and thus loss of oxygen-sensitive taxa. The finding that TN was the best predictor for profundal community changes, and that temperature, water color and NAO were also important supports this conjecture. Moreover, the population increase of *Chaoborus flavicans* in profundal habitats, a taxon that is relatively mobile and hence can easily avoid hypoxia, also lends support to this conjecture. A shift or changes in spring temperature as result of warmer winters in the early years of the study (high  $NAO_{winter}$  indices through 1988-1995) may also have resulted in changes of the timing and prolongation of summer stratification. An earlier onset of the spring stratification, due to faster temperature rise in spring temperature, has been found to influence the nutrient cycling (Schindler *et al.* 1990; Agbeti & Smol 1995). Moreover, prolonged summer stratification periods caused by climate warming are predicted to result in increased hypolimnetic anoxia (Magnuson *et al.* 1997). If the sublittoral habitat is periodically situated under the summer thermocline, lower oxygen concentrations and/or lower ambient temperatures may then negatively affect sublittoral communities.

Many of the factors discussed above suggest that climate change may have substantial influence on aquatic ecosystems and might affect or even confound recovery processes indirectly (e.g. via increased inputs of nutrients or organic matter) and directly (e.g. via changes in temperature).

The increasing trends in lake acidity, phytoplankton and littoral community composition indicate that lakes are showing signs of recovery. However, with the exception of littoral

macroinvertebrate diversity, all other chemical and biological indicators did not differ between 1988 and 2003 indicating clear differences between acidified and reference lakes. The negative changes noted for sublittoral and profundal communities indicate that choice of not only organism but also habitat may be important to detecting biological recovery. Our study indicates that sublittoral and profundal macroinvertebrate communities may be more influenced by climate-related variables acting on habitat quality (e.g. ambient oxygen and temperature) than by changes in lake acidity. Moreover, the finding that both lake chemistry and biology were correlated to a number of climate related variables supports the conjecture that climate-related factors might confound assessment of recovery. In summary, our findings indicate that recovery is a complex process influenced by multiple spatial and temporal factors. These findings lend support to the use of multiple organism groups and trophic levels to better detect and understand the processes and changes within the lake ecosystem.

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Table 1: Physico-chemical characteristics of the reference (r; n = 6) and the acidified (a; n = 4) lakes. Chemistry variables with mean values (and 10th and 90th percentiles in parenthesis). TN = total nitrogen; TP = total phosphorous.

Lake name/type	Area [km <sup>2</sup> ]	Mean depth [m]	pH	Conductivity [ms m <sup>-1</sup> ]	Alkalinity [meq L <sup>-1</sup> ]	TN [µg L <sup>-1</sup> ]	TP [µg L <sup>-1</sup> ]	Water colour [420 5cm <sup>-1</sup> ]
<b>Reference</b>								
Median R <sup>2</sup> *			0.31	0.5				0.45
Median slope*			0.01	-0.009				0.003
Allgjuttern (r)	0.5	11.4	6.6 (6.5-6.7)	5.2 (4.5-6.5)	0.07 (0.06-0.08)	367.2 (293.8-413.2)	8.5 (5.3-15.8-)	0.05 (0.03-0.06)
Fiolen (r)	1.7	3.8	6.4 (6.1-6.7)	6.0 (5.6-6.6)	0.05 (0.03-0.07)	494.1 (417.7-591.7)	12.1 (9.5-15.4)	0.04 (0.03-0.07)
Fräcksjön (r)	0.3	6.0	6.4 (6.2-6.5)	7.5 (5.7-9.3)	0.06 (0.04-0.07)	461.4 (387-560.1)	10.4 (7.9-12.5)	0.10 (0.07-0.15)
Stensjön (r)	0.5	4.2	6.3 (6.1-6.5)	1.8 (1.6-2)	0.04 (0.03-0.06)	293 (244.1-359.1)	8.8 (6.1-11.8)	0.10 (0.07-0.13)
Stora Envättern (r)	0.4	5.0	6.5 (6.5-6.6)	4.3 (3.7-4.6)	0.06 (0.04-0.07)	416.4 (359.6-467.1)	9.7 (6.8-12.2)	0.06 (0.04-0.10)
Stora Skärsjön (r)	0.3	3.8	6.8 (6.6-6.9)	8.2 (7.3-8.9)	0.11 (0.10-0.13)	401.6 (326.5-464.5)	9.8 (6.7-13.3)	0.04 (0.03-0.07)
<b>Acidified</b>								
Median R <sup>2</sup> *			0.39	0.52				0.49
Median slope*			0.02	-0.02				0.007
Brunnsjön (a)	0.1	5.3	5.4 (5.2-5.6)	7.0 (5.6-8.8)	0.001 (-0.008-0.012)	663.4 (541.4-792.7)	13.1 (9.7-17.3)	0.35 (0.16-0.57)
Härsvatten (a)	0.2	5.7	4.6 (4.4-4.8)	6.6 (4.8-8.3)	-0.03 (-0.06-0.0)	333.2 (290.7-402)	5.2 (3.3-7.7)	0.01 (0.005-0.019)
Övre Skärsjön (a)	1.7	5.7	5.5 (5.1-5.7)	3.1 (2.4-3.4)	0.003 (-0.002 0.01)	392.7 (348.6-438.9)	7.4 (6.0-9.0)	0.12 (0.08-0.19)
Rotehogstjärnen (a)	0.2	3.4	5.4 (5.1-5.6)	5.5 (4.0-7.2)	0.003 (-0.008-0.014)	445.3 (402-539.2)	14.4 (12.3-16)	0.21 (0.13-0.30)

Table 2: Differences in chemistry, phytoplankton and benthic invertebrate diversity and taxon richness means (+ 1SD) between reference (r) and acidified (a) lakes over all 16 years (grand mean), in the first and the last sampling year (1988/89 and 2003) and differences of first and last year within lake types (1988 vs. 2003). Asterisks indicating significant differences (two-sided t-test) at \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .

	Grand mean		1988		2003		1988 vs. 2003	
	r	a	r	a	r	a	r	a
<u>Chemistry</u>								
pH	6.49 ± 0.19***	5.22 ± 0.42	6.34 ± 0.18***	4.96 ± 0.38	6.62 ± 0.19**	5.42 ± 0.40	*	n.s.
Alkalinity	0.06 ± 0.03***	-0.005 ± 0.02	0.05 ± 0.02**	0.0005 ± 0.0009	0.08 ± 0.04**	-0.004 ± 0.01	n.s.	n.s.
Conductivity	5.48 ± 2.20	5.55 ± 1.85	5.42 ± 2.23	5.24 ± 1.70	4.7 ± 1.94	4.38 ± 1.75	n.s.	n.s.
TN	405.61 ± 81**	458.66 ± 137.71	365.66 ± 90.30	434.08 ± 116.51	406.31 ± 75.72	413.13 ± 105.24	n.s.	n.s.
TP	9.88 ± 2.62	10.05 ± 4.20	8.86 ± 1.06	9.88 ± 4.85	9 ± 5.16	8.03 ± 3.94	n.s.	n.s.
Water colour	0.07 ± 0.03***	0.17 ± 0.14	0.07 ± 0.02	0.19 ± 0.16	0.08 ± 0.03	0.18 ± 0.13	n.s.	n.s.
<u>Simpson's diversity</u>								
Phytoplankton	3.34 ± 0.14***	2.78 ± 0.43	3.08 ± 0.26*	2.54 ± 0.33	3.64 ± 0.17**	3.01 ± 0.20	**	n.s.
Littoral	15.28 ± 4.61***	10.65 ± 4.39	10.05 ± 2.09***	5.99 ± 1.15	19.19 ± 3.37	14.96 ± 5.86	**	*
Sublittoral	10.01 ± 3.78***	5.64 ± 2.60	11.44 ± 4.57	7.22 ± 2.83	7.68 ± 2.71	6.36 ± 3.63	n.s.	n.s.
Profundal	3.47 ± 1.68	2.43 ± 1.10	4.58 ± 1.24	3.03 ± 1.21	2.56 ± 1.41	2.03 ± 0.42	*	n.s.
<u>Taxon richness</u>								
Phytoplankton	5.92 ± 0.79***	4.67 ± 1.39	4.67 ± 1.03	3.25 ± 1.26	6.17 ± 0.41	5.25 ± 1.70	**	n.s.
Littoral	32.9 ± 9.5***	21.35 ± 9.15	10.05 ± 2.09	3.03 ± 1.21	39.0 ± 6.87	27.5 ± 12.04	**	n.s.
Sublittoral	14.72 ± 5.9***	8.48 ± 3.42	18.00 ± 6.44	9.67 ± 3.51	10.33 ± 4.03	11.0 ± 4.76	*	n.s.
Profundal	5.05 ± 2.69	3.60 ± 1.77	4.58 ± 1.24	3.03 ± 1	3.17 ± 1.72	3.5 ± 1.0	**	n.s.

Table 3: Results of the regression analyses of phytoplankton taxon richness and Simpson diversity versus time of the reference (n = 6) and the acidified (n = 4) lakes. The first row of each lake is the coefficient of determination ( $R^2$  [year<sup>-1</sup>], and root mean square error in parenthesis) and the third row is the slope ([year<sup>-1</sup>]  $\pm$  1 SE in parenthesis).  $P$  = probability = <0.05 is considered significant and shown in bold.

	Taxon richness		Simpson diversity		
Lake/type		$P$		$P$	
<b>Reference</b>					
Median $R^2$ *		0.28		0.31	
Median slope*		0.09		0.037	
Allgjuttern	$R^2$	0.36 (0.71)	< <b>0.01</b>	0.26 (0.25)	< <b>0.05</b>
	slope	0.12 (0.04)		0.03 (0.01)	
Fiolen		0.34 (0.58)	< <b>0.01</b>	0.54 (0.18)	< <b>0.001</b>
		0.09 (0.03)		0.04 (0.01)	
Fräcksjön		0.13 (0.54)	0.09	0.07 (0.21)	0.16
		0.05 (0.03)		0.02 (0.01)	
Stensjön		-0.03 (0.58)	0.44	0.36 (0.31)	< <b>0.01</b>
		0.03 (0.03)		0.05 (0.02)	
Stora Envättern		0.28 (0.66)	< <b>0.05</b>	0.37 (0.21)	< <b>0.01</b>
		0.09 (0.04)		0.04 (0.01)	
Stora Skärsjön		0.11 (0.59)	0.12	0.20 (0.32)	< <b>0.05</b>
		0.05 (0.03)		0.04 (0.02)	
<b>Acidified</b>					
Median $R^2$ *		0.39		0.28	
Median slope*		0.12		0.044	
Brunnsjön		0.32 (0.73)	< <b>0.05</b>	0.49 (0.24)	< <b>0.01</b>
		0.11 (0.04)		0.05 (0.01)	
Härsvatten		0.49 (0.55)	< <b>0.01</b>	0.24 (0.29)	< <b>0.05</b>
		0.12 (0.03)		0.04 (0.02)	
Övre Skärsjön		0.47 (0.61)	< <b>0.01</b>	0.09 (0.29)	0.14
		0.13 (0.03)		0.02(0.02)	
Rotehogstjärnen		0.30 (1.35)	< <b>0.01</b>	0.32 (0.39)	< <b>0.05</b>
		0.20 (0.07)		0.06 (0.02)	

\* refer to Fig. 3 a-d

Table 4: Trends of the phytoplankton groups (biovolume [ $\text{mm L}^{-1}$ ]) found in reference and acidified lakes. Shown are numbers of times (n), the algae group showed a significant trend, and the mean of the slopes ( $\pm$  SD) for these groups.

Note: shown are only species showing significant trends.

Algae group	Reference lakes		Acidified lakes	
	n	Slope	n	Slope
<i>Cyanophyceae</i>	4	$0.11 \pm 0.02$	–	–
<i>Cryptophyceae</i>	4	$0.09 \pm 0.02$	3	$0.10 \pm 0.03$
<i>Dinophyceae</i>	2	$0.11 \pm 0.07$	2	$0.13 \pm 0.01$
<i>Crysophyceae</i>	5	$0.10 \pm 0.04$	2	$0.10 \pm 0.04$
<i>Bacillariophyceae</i>	2	$0.08 \pm 0.02$	2	$0.13 \pm 0.03$
<i>Chlorophyceae</i>	5	$0.10 \pm 0.02$	1	0.12

Table 5: Results of the regression analyses of macroinvertebrate taxon richness and Simpson diversity vs. time for each habitat of the reference (n = 6) and the acidified (n = 4) lakes. The first row of each lake is the coefficient of determination ( $R^2$ , and root mean square error in parenthesis) and the third row is the slope ( $\pm 1$  SE in parenthesis).  $P = \text{probability} = <0.05$  is considered significant and shown in bold.

Lake/type	Taxon richness						Simpson diversity					
	Littoral	<i>P</i>	Sublittoral	<i>P</i>	Profundal	<i>P</i>	Littoral	<i>P</i>	Sublittoral	<i>P</i>	Profundal	<i>P</i>
<b>Reference</b>												
Median $R^2$ *	0.15		0.34		0.26		0.24		0.28		0.15	
Median slope*	0.97		-0.50		-0.17		0.52		-0.32		-0.07	
Allgjuttern	$R^2$ -0.07 (7.56)	0.88	0.19 (4.54)	0.06	0.25 (1.20)	<b>&lt; 0.05</b>	-0.04 (4.63)	0.51	0.11 (3.37)	0.13	0.08 (0.79)	0.17
	Slope 0.06 (0.41)		-0.56 (0.27)		-0.17 (0.08)		-0.17 (0.25)		-0.33 (0.20)		-0.07 (0.05)	
Fiolen	0.44 (8.60)	<b>&lt; 0.01</b>	0.49 (4.46)	<b>&lt; 0.01</b>	0.39 (2.52)	<b>&lt; 0.01</b>	0.16 (4.68)	0.06	0.45 (2.54)	<b>&lt; 0.01</b>	0.17 (1.53)	0.6
	1.67 (0.47)		-1.01 (0.27)		0.44 (0.14)		0.50 (0.25)		-0.53 (0.15)		0.17 (0.08)	
Fräcksjön	0.18 (10.48)	0.06	0.48 (1.95)	<b>&lt; 0.01</b>	0.19 (1.43)	0.051	0.31 (3.54)	<b>&lt; 0.05</b>	0.59 (1.14)	<b>&lt; 0.001</b>	0.13 (0.77)	0.09
	1.19 (0.57)		-0.43 (0.12)		-0.17 (0.08)		0.54 (0.19)		-0.31 (0.07)		-0.07 (0.04)	
Stora Skärsjön	0.12 (7.21)	0.11	0.71 (4.18)	<b>&lt; .0001</b>	0.27 (1.48)	<b>&lt; 0.05</b>	0.46 (2.99)	<b>&lt; 0.01</b>	0.67 (2.86)	<b>&lt; .0001</b>	0.16 (1.02)	0.07
	0.74 (0.43)		-1.48 (0.25)		-0.20 (0.08)		0.64 (0.18)		-0.94 (0.17)		-0.11 (0.06)	
Stensjön	0.58 (6.59)	<b>&lt; 0.001</b>	-0.06 (4.07)	0.66	-0.02 (1.12)	0.41	0.68 (3.02)	<b>&lt; 0.0001</b>	-0.06 (2.86)	0.62	-0.05 (0.71)	0.60
	1.66 (0.36)		-0.11 (0.24)		-0.05 (0.06)		0.94 (0.16)		-0.09 (0.17)		0.02 (0.04)	
Stora Envättern	0.05 (7.66)	0.20	0.01 (5.10)	0.31	0.55 (1.42)	<b>&lt; 0.001</b>	-0.05 (2.85)	0.60	-0.06 (3.83)	0.56	0.51 (1.22)	<b>&lt; 0.01</b>
	0.56 (0.42)		-0.40 (0.38)		-0.34 (0.08)		0.08 (0.16)		-0.17 (0.28)		-0.27 (0.07)	

Table 5: continued

Lake	Taxon richness						Simpson diversity					
	Littoral	<i>P</i>	Sublittoral	<i>P</i>	Profundal	<i>P</i>	Littoral	<i>P</i>	Sublittoral	<i>P</i>	Profundal	<i>P</i>
<b>Acidified</b>												
Median $R^2$ *	0.32		-0.02		-0.03		0.34		-0.03		0.08	
Median slope*	0.88		0		-0.03		0.5		-0.09		-0.03	
Brunnsjön	$R^2$ 0.37 (6.29)	< <b>0.01</b>	-0.04 (2.32)	0.46	-0.06 (0.99)	0.75	0.25 (3.8)	< <b>0.05</b>	0.02 (2.10)	0.28	0.05 (0.35)	0.21
	Slope 1.06 (0.34)		-0.13 (0.17)		0.02 (0.05)		0.51 (0.21)		-0.18 (0.16)		0.02 (0.02)	
Härsvatten	0.27 (3.63)	< <b>0.05</b>	0.40 (1.58)	< <b>0.05</b>	-0.04 (1.51)	0.50	0.29 (2.31)	< <b>0.05</b>	0.10 (1.19)	0.15	-0.06 (1.18)	0.78
	0.49 (0.19)		0.33 (0.11)		0.06 (0.08)		0.32 (0.13)		0.13 (0.08)		0.02 (0.06)	
Övre Skärsjön	0.27 (4.95)	< <b>0.05</b>	-0.04 (3.24)	0.52	-0.01 (1.3)	0.38	0.57 (2.0)	< <b>0.001</b>	-0.07 (2.63)	0.81	0.10 (0.92)	0.14
	0.69 (0.27)		0.13 (0.2)		-0.07 (0.08)		0.49 (0.11)		-0.04 (0.16)		-0.08 (0.05)	
Rotehogstjärnen	0.60 (4.64)	< <b>0.01</b>	0.01 (2.92)	0.31	0.10 (2.06)	0.12	0.42 (3.53)	< <b>0.01</b>	0.15 (1.15)	0.09	0.36 (0.82)	< <b>0.01</b>
	1.14 (0.25)		-0.18 (0.17)		-0.18 (0.11)		0.62 (0.19)		-0.13 (0.07)		-0.14 (0.04)	

\* refer to Fig. 4 a-d

Table 6: Trends in abundance of the 10 most abundant (acidified profundal: range from 2 to 10) species found in reference and acidified lakes. Shown are numbers of times (n), the species showing a significant trend, and the mean of the slopes ( $\pm$  1SD) for these species.

Note: shown are only species showing significant trends.

Reference lakes								
Littoral			Sublittoral			Profundal		
Species	n	Slope	Species	n	Slope	Species	n	Slope
<i>Asellus aquaticus</i>	1	1.54	<i>Asellus aquaticus</i>	4	-0.46 $\pm$ 0.23	<i>Chaoborus flavicans</i>	2	0.20 $\pm$ 0.08
<i>Caenis horaria</i>	2	0.44 $\pm$ 0.02	<i>Ephemera vulgata</i>	1	-0.14	<i>Chironomus anthracinus</i>	1	-0.05
<i>Caenis luctuosa</i>	1	0.38	<i>Leptophlebia vespertina</i>	1	-0.28	<i>Mysis relicta</i>	1	-0.10
<i>Centroptilum luteolum</i>	1	0.33	<i>Oxyethira sp.</i>	1	-0.18	<i>Pisidium sp.</i>	2	-0.46 $\pm$ 0.35
<i>Ephemera vulgata</i>	1	0.69	<i>Pagastiella orophila</i>	3	-0.30 $\pm$ 0.07	<i>Procladius sp.</i>	1	-0.14
<i>Gyraulus albus</i>	1	0.66	<i>Pisidium sp.</i>	3	-0.31 $\pm$ 0.08	<i>Sergentia coracina</i>	1	-0.41
<i>Hydroptila sp.</i>	1	0.89	<i>Polypedilum sp.</i>	1	-0.26	<i>Zalutschia zalutschicola</i>	1	-0.21
<i>Lauterborniella agrayloides</i>	1	0.46	<i>Procladius sp.</i>	2	-0.24 $\pm$ 0.06			
<i>Oxyethira sp.</i>	1	0.32	<i>Pseudochironomus prasinatus</i>	1	-0.32			
<i>Paratanytarsus sp.</i>	1	0.51	<i>Sialis lutaria</i>	1	0.21			
<i>Pisidium sp.</i>	3	0.93 $\pm$ 0.62	<i>Sialis sp.</i>	1	-0.25			
<i>Psectrocladius sp.</i>	1	0.87	<i>Zalutschia zalutschicola</i>	2	-0.42 $\pm$ 0.18			
<i>Tanytarsus sp.</i>	2	0.27 $\pm$ 0.17						

Table 6: continued

Acidified lakes								
Littoral			Sublittoral			Profundal		
Species	n	Slope	Species	n	Slope	Species	n	Slope
<i>Procladius sp.</i>	1	0.35	<i>Procladius sp.</i>	1	0.18 ± 0.09	<i>Chaoborus flavicans</i>	1	0.35
<i>Pisidium sp.</i>	1	0.40	<i>Sergentia coracina</i>	1	0.30	<i>Polypedium sp.</i>	1	-0.06
<i>Dicrotendipes sp.</i>	1	0.34	<i>Chaoborus flavicans</i>	1	0.25	<i>Pagastiella orophila</i>	1	-0.11
<i>Asellus aquaticus</i>	2	0.8 ± 0.3	<i>Sialis sp.</i>	1	-0.28	<i>Chironomus anthracinus</i>	1	-0.28
<i>Leptophlebia vespertina</i>	1	0.20	<i>Paramerina sp.</i>	1	0.75	<i>Chironomus plumosus</i>	1	-0.25
<i>Sialis lutaria</i>	1	0.15	<i>Zalutschia zalutschicola</i>	1	0.22	<i>Zalutschia zalutschicola</i>	1	-0.38
<i>Sialis sp.</i>	1	-0.25	<i>Pagastiella orophila</i>	1	0.18			
<i>Tanytarsus sp.</i>	1	0.33	<i>Stictochironomus rosenschoeldi</i>	1	0.52			
<i>Pagastiella orophila</i>	1	0.34						

Table 7: Relative and mean (in parenthesis) slopes ( $\pm 1SD$ ) of selected indicators from four acidified and six reference lakes sampled from 1988 until 2003. Relative regression slopes were calculated as a proportional change from year 1 (i.e. annual mean values were divided by the mean value of 1988). Letters show significant differences using Tukey-Kramer HSD tests on relative slopes.

Lake type/variable	Acidity	Taxon richness	Diversity
<b>Acidified</b>			
pH	0.0047 $\pm$ 0.0016 (0.0218 $\pm$ 0.0089)		BC B
phytoplankton		0.0445 $\pm$ 0.0097 (0.138 $\pm$ 0.042)	AB 0.0174 $\pm$ 0.0068 (0.043 $\pm$ 0.0171)
littoral benthos		0.0645 $\pm$ 0.0293 (0.845 $\pm$ 0.307)	A 0.0851 $\pm$ 0.0287 (0.488 $\pm$ 0.120)
sublittoral benthos		-0.0048 $\pm$ 0.0204 (0.038 $\pm$ 0.238)	C -0.0139 $\pm$ 0.0135 (-0.055 $\pm$ 0.136)
profundal benthos		-0.0069 $\pm$ 0.0190 (-0.043 $\pm$ 0.1066)	C -0.0099 $\pm$ 0.0239 (-0.045 $\pm$ 0.084)
<b>Reference</b>			
pH	0.0019 $\pm$ 0.0021 (0.006 $\pm$ 0.016)		BC BC
phytoplankton		0.0176 $\pm$ 0.0125 (0.072 $\pm$ 0.034)	AB 0.0124 $\pm$ 0.0041 (0.0367 $\pm$ 0.0103)
littoral benthos		0.0489 $\pm$ 0.0364 (0.98 $\pm$ 0.64)	A 0.0443 $\pm$ 0.0465 (0.422 $\pm$ 0.401)
sublittoral benthos		-0.0339 $\pm$ 0.0160 (-0.665 $\pm$ 0.496)	D -0.0327 $\pm$ 0.0162 (-0.400 $\pm$ 0.307)
profundal benthos		-0.0306 $\pm$ 0.0159 (-0.082 $\pm$ 0.272)	CD -0.0222 $\pm$ 0.0184 (-0.055 $\pm$ 0.146)

Table 8: Summary of the results of stepwise multiple regressions of CA axis 1 scores, Simpson's diversity and taxon richness of phytoplankton and macroinvertebrates (littoral, sublittoral, profundal) with eight environmental variables. Numbers show the number of times the explanatory variable was selected as significant predictors for six reference (r) and four acidified (a) lakes. The last column shows the combined number with the best predictor for each habitat shown in bold.  $NAO_{winter}$  = NAO winter index (December-March)

Lake type/habitat/variable	CA axis 1 scores		Simpson's diversity		Taxon richness		$\Sigma$
	r	a	r	a	r	a	
<b>Pelagial</b>							
pH	2	1	3	2	3	1	<b>12</b>
Alkalinity	2	0	1	2	2	2	9
Conductivity	2	3	3	1	2	0	11
TP	1	2	1	0	0	0	4
TN	1	0	2	1	2	1	7
Water colour	1	0	1	2	1	4	9
Temperature	1	2	2	2	1	2	10
$NAO_{winter}$	2	1	1	1	3	0	8
<b>Littoral</b>							
pH	3	2	3	3	3	2	<b>16</b>
Alkalinity	2	2	1	1	1	3	10
Conductivity	1	2	2	0	3	1	9
TP	1	3	5	0	1	0	10
TN	1	2	3	0	2	1	9
Water colour	0	2	1	2	2	1	8
Temperature	0	1	1	3	2	4	11
$NAO_{winter}$	2	0	2	1	0	1	6
<b>Sublittoral</b>							
pH	1	2	3	1	1	1	9
Alkalinity	1	3	1	2	3	1	11
Conductivity	4	0	0	0	0	1	5
TP	3	0	3	1	3	2	12
TN	1	2	0	0	1	1	5
Water colour	2	2	5	1	4	1	<b>15</b>
Temperature	2	1	1	0	2	1	7
$NAO_{winter}$	1	2	0	1	1	1	6
<b>Profundal</b>							
pH	1	1	2	1	4	1	10
Alkalinity	2	1	2	0	2	3	10
Conductivity	1	3	1	0	1	1	7
TP	0	1	2	0	2	2	7
TN	2	1	4	1	6	1	<b>15</b>
Water colour	1	3	3	1	2	1	11
Temperature	2	1	2	1	2	1	9
$NAO_{winter}$	1	2	0	2	1	2	8

## Figure legends

Figure 1: Location of the six reference and four acidified (in italics) lakes. 20 refers to the arctic/alpine biogeographic region, 22 refers to the boreal coniferous forest region and 14 refers to the mixed forest region according to (Illies 1966).

Figure 2: Box plots of slopes and coefficients of determinants ( $R^2$ ) of the regression analyses of pH (a & b), conductivity (c & d), and water colour (e & f) versus year (1988-2003). Different letters indicate significant differences between lakes. Numbers in parenthesis are number of significant regressions out of total regressions.

Figure 3: Box plots of slopes and coefficients of determinants ( $R^2$ ) of the regression analyses of phytoplankton taxon richness (a & b) and Simpson diversity (c & d) versus year (1988-2003). Different letters indicate significant differences between lakes. Numbers in parenthesis are numbers of significant regressions out of total regressions.

Figure 4: Box plots of slopes and coefficients of determinants ( $R^2$ ) of the regression analyses of macroinvertebrate taxon richness (a & b) and Simpson diversity (c & d) versus year (1988-2003). rL, rSL, rP = reference littoral, reference sublittoral, reference profundal; aL, aSL, aP = acidified littoral, acidified sublittoral, acidified profundal. Different letters indicate significant differences between habitats/lakes. Numbers in parenthesis are numbers of significant regressions out of total regressions.

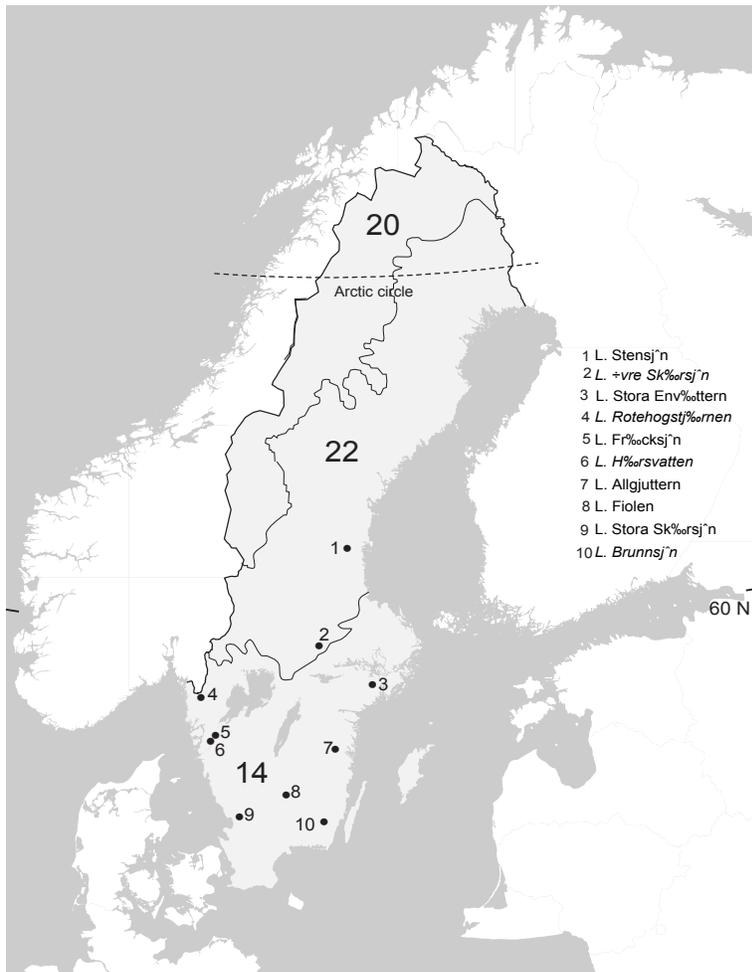


Figure 1

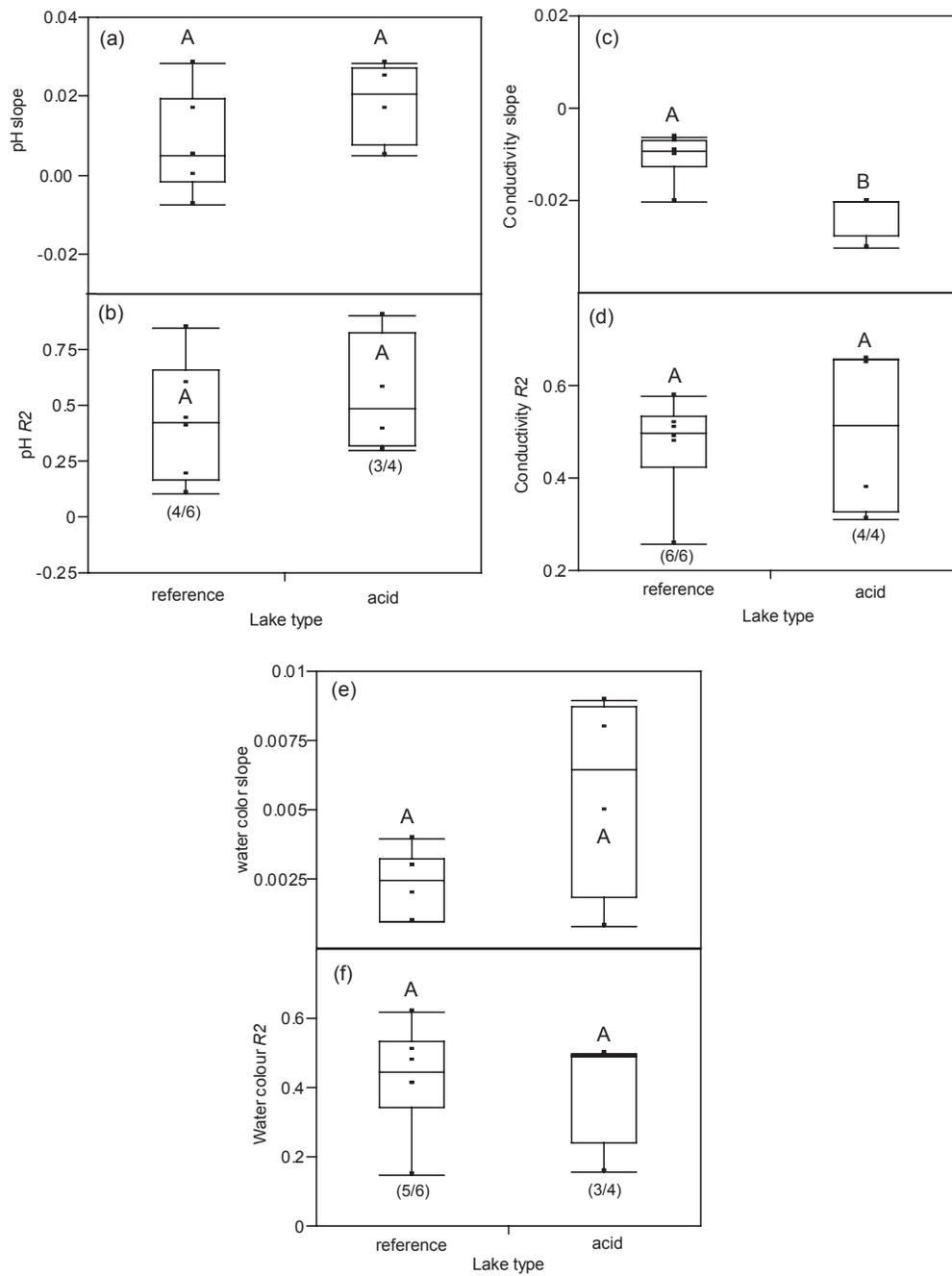


Figure 2

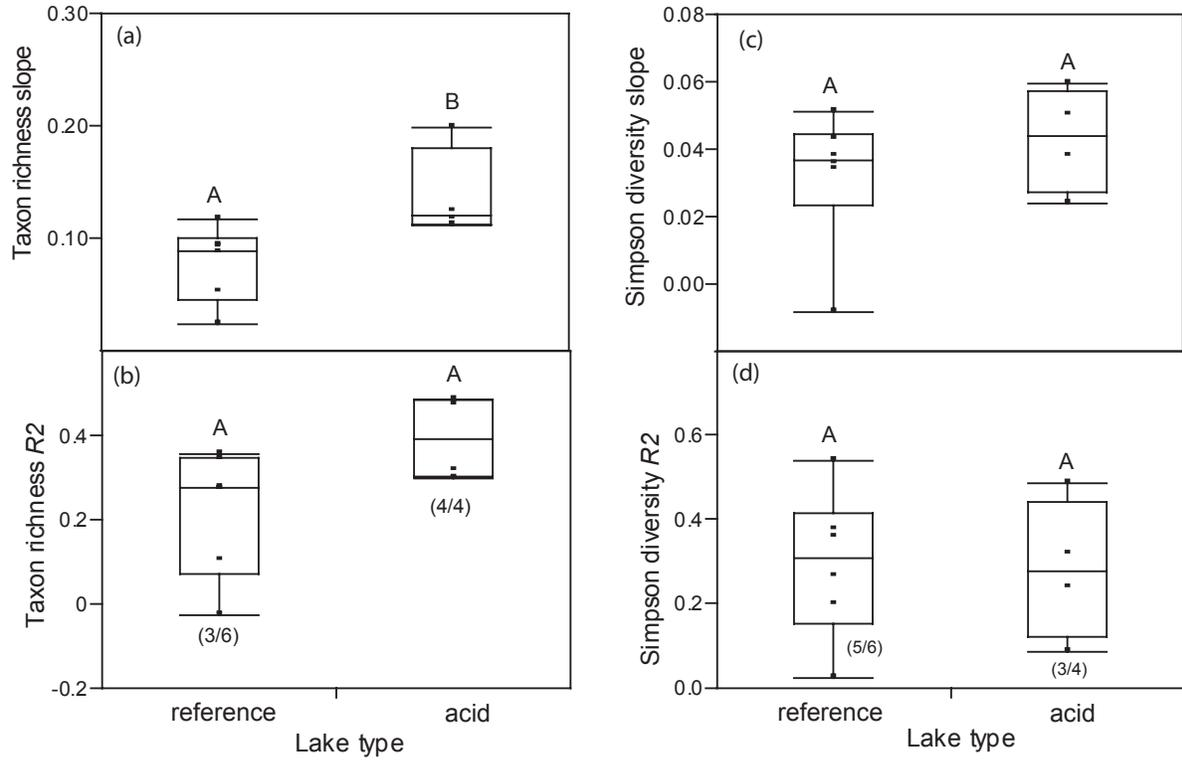


Figure 3

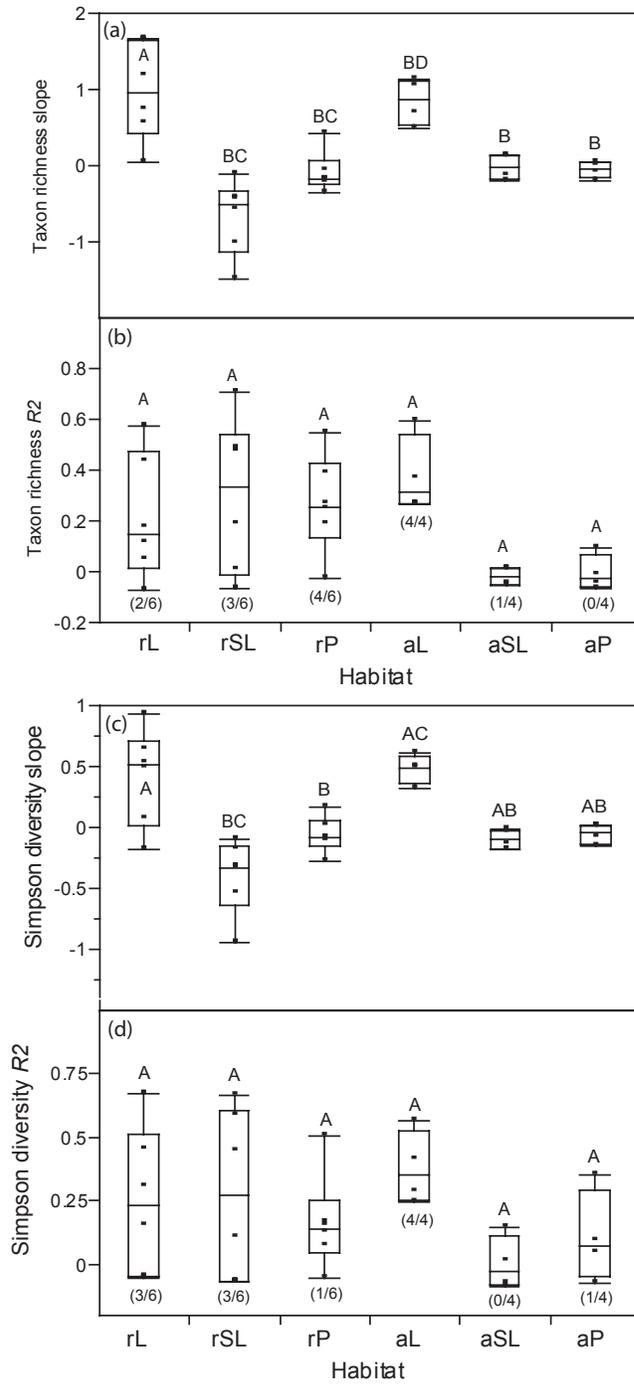


Figure 4